

AWARD NUMBER:

W81XWH-14-1-0452

TITLE:

"Smart, Injury-Triggered Therapy for Ocular Trauma

PRINCIPAL INVESTIGATOR:

Dr. William Jia

CONTRACTING ORGANIZATION:

University of British Columbia, Vancouver V6T 1Z3

REPORT DATE:

October 2015

TYPE OF REPORT:

Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE October 2015		2. REPORT TYPE Annual		3. DATES COVERED 15 Sep 2014 - 14 Sep 2015	
4. TITLE AND SUBTITLE Smart, Injury-Triggered Therapy for Ocular Trauma				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-14-1-0452	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) William Jia Max Cynader Joanne Matsubara Ljubomir Kojic Jing Cui Xuexian Bu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of British Columbia Department of Surgery 2194 Health Sciences Mall Vancouver, BC V6T 1Z3 Canada				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Traumatic eye injury (TEI) is one of the leading causes of monocular blindness in military personnel and young males worldwide. This profound and frequently irreversible posttraumatic loss of vision has a poor prognosis due to retinal cell death, scar formation, and lack of functional regeneration. Proliferative vitreoretinopathy (PVR), a form of intraocular fibrosis, is often the primary reason for the loss of vision after ocular trauma, and frequently occurs after blunt trauma and open globe injuries caused by penetration, rupture, perforation, and presence of intraocular foreign bodies as well as after retinal re-attachment surgery. We genetically engineered "protease activity sensor" (PAS) as chimeric transmembrane protein that can respond to increase in metalloproteinase activity by shedding/releasing tagged-ectodomains in the vicinity of affected cells after traumatic eye injury and induction of PVR. We demonstrated that upon infection with AAV carrying our construct, HEK293 cells and neurons in culture expressed the engineered HA-tagged PAS proteins. Their HA-tagged ectodomains were detected in the extracellular medium within minutes following stimulation with ionomycin and glutamate respectively. We used a rabbit PVR model of ocular trauma in which autologous blood was injected into the vitreous cavity of one eye after a surgical incision through the pars plana. Following the eye injury, we have isolated the eyeballs from experimental animals to evaluate the stage of PVR and the expression and activation of MPs after the ocular trauma.					
15. SUBJECT TERMS Trauma, Injury, Eye, Retina, PVR, Neuroinflammation, Proteases, Metalloproteinases, Cell death, Gene Therapy					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code)

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1. INTRODUCTION:

Traumatic eye injury (TEI) is one of the leading causes of monocular blindness in military personnel and young males worldwide (Negrel and Thylefors, 1998). This profound and frequently irreversible posttraumatic loss of vision has a poor prognosis due to retinal cell death, scar formation, and lack of functional regeneration. Proliferative vitreoretinopathy (PVR), a form of intraocular fibrosis, is often the primary reason for the loss of vision after ocular trauma, and frequently occurs after blunt trauma and open globe injuries caused by penetration, rupture, perforation, and presence of intraocular foreign bodies as well as after retinal re-attachment surgery (Mietz et al., 1994; Cardillo et al., 1997). Many of the pathological processes driving PVR can be traced to the local, inflammatory processes that trigger several downstream mechanisms including the activation of metalloproteinases (MPs), that facilitate extracellular matrix (ECM) remodeling, proliferation and migration of inflammatory cell, inducing formation and contraction of periretinal membranes resulting in PVR and retinal detachment (Symeonidis et al., 2011). Since the activation of MPs is an immediate event following the primary injury, the instantaneous and specific release of therapeutics at the site of injury may limit the extent of inflammation, provide sufficient cytoprotection and reduce the probability of PVR following the TEI.

2. KEYWORDS:

Trauma, Injury, Eye, Retina, PVR, Neuroinflammation, Proteases, Metalloproteinases, Cell death, Gene Therapy

3. ACCOMPLISHMENTS:

Major goals of the project

1. To obtain animal protocol approval from the local Animal Care Committee (ACC) at University of British Columbia (UBC), equivalent to the Institutional Animal Care and Use Committee (IACUC) in US, and then to obtain the regulatory approval for use of animal subjects from the Animal Care and Use Review Office (ACURO)
2. To evaluate the expression and activation of MPs and plasma membrane shedding in the eye after ocular trauma
3. To develop of transmembrane proteins that will respond to the increase of MPs activity in animal model of PVR
4. To evaluate two therapeutic-PAS (Protease Activity Sensors) in PVR animal model of ocular trauma

What was accomplished under these goals?

The major activities during the first year of the project were focused on developing and evaluating a rabbit model of PVR, as an experimental model of ocular trauma, and the developing of trauma-induced gene therapy and its delivery after ocular injury.

Specifically, to initiate the work on the animal model we have developed animal protocol and applied for its regulatory approval by the Animal Care Committee (ACC) at the University of British Columbia (UBC), a Canadian equivalent to the Institutional Animal Care and Use Committee

(IACUC), and subsequently by the Animal Care and Use Review Office (ACURO) at DoD. After obtaining the initial approval for our animal model, we have obtained regulatory approvals from IACUC and ACURO for the use of AAV viruses for intraocular delivery of gene therapy designed to alleviate the effects of ocular trauma.

The protocol MR130584 entitled, "Mechanisms of Ocular Disease," IACUC protocol number A14-0320 was approved by the USAMRMC Animal Care and Use Review Office (ACURO) for the use of rabbits. We expect that a single animal use research protocols will be required to complete the Statement of Work (SOW). The protocol will be amended when new procedures are required and the amendments will be submitted for approval to local IACUC and DoD ACURO prior to starting any animal work.

Approval of the animal model of ocular trauma was necessary to study the role of metalloproteinases in ocular trauma, including their expression, activation and their involvement in the pathophysiology of the injury. We have also initiated the development of transmembrane proteins that will respond to the increase of metalloproteinase activity in animal model of PVR and will be used as therapeutic tools in ocular trauma. Their therapeutic potential have been validated *in vitro*.

Significant results or key outcomes

We genetically engineered "protease activity sensor" (PAS) as chimeric transmembrane protein that can respond to increase in metalloproteinase activity by shedding/releasing tagged-ectodomains in the vicinity of affected cells after traumatic eye injury and induction of PVR.

These membrane spanning PAS construct consists of 2 reporter elements, including green fluorescent protein (GFP) (Tsien, 1998), and a hemagglutinin A (HA) tags (Zhao et al., 2013). A protease cleavage site, engineered to be sensitive to injury/inflammation activated membrane associated proteases of the ADAM and MMP families (Candelario-Jalil et al., 2009; Rosenberg, 2009) is located just extracellular to the membrane spanning domain of the construct. We confirmed that extracellular metalloproteinases are activated within minutes under various adverse, including excitotoxicity and inflammation.

We demonstrated that upon infection with AAV carrying our construct, HEK293 cells in culture expressed the engineered HA-tagged PAS proteins (Fig. 1A). Their HA-tagged ectodomains were detected in the extracellular medium within minutes following stimulation with ionomycin (Fig. 1B).

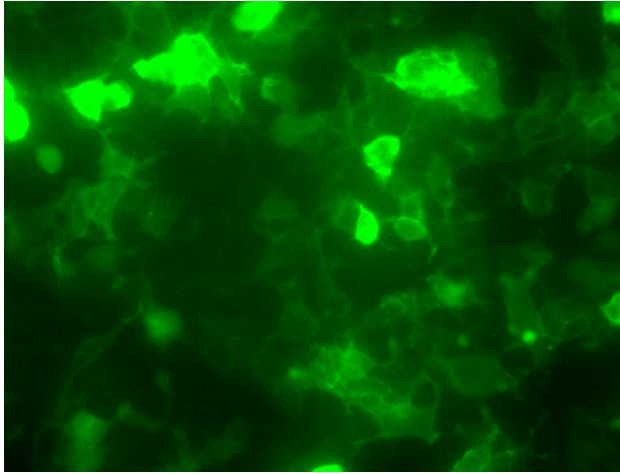
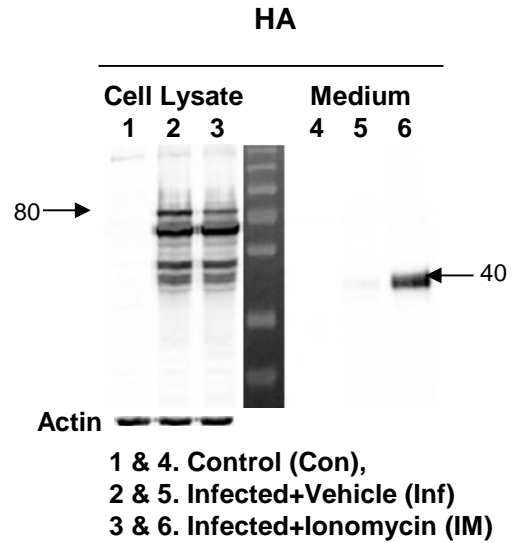
A**B**

Fig. 1. The effects of ionomycin on the shedding of chimeric fractalkine constructs from HEK293 cells *in vitro*. **(A)** HEK293 cell cultures were transduced with the AAV virus carrying an N-terminal-HA-tagged fractalkine as a transgene. **(B)** Cells were treated with ionomycin (IM; 1 μ mol/L for 20 min). The cell lysate and conditioned media were assayed for the release of HA-tagged fragments using Western blot (WB). There was a small reduction of the full length (80 kDa) protein (lane 3) in HEK293 cells accompanied with a release of soluble extracellular domain (40 kDa) in the medium (lane 6), confirming the cleavage event. HEK293 cells expressing the HA tagged construct have demonstrated a relatively low background cleavage activity (lane 5) with a robust release after ionomycin stimulation (lane 6).

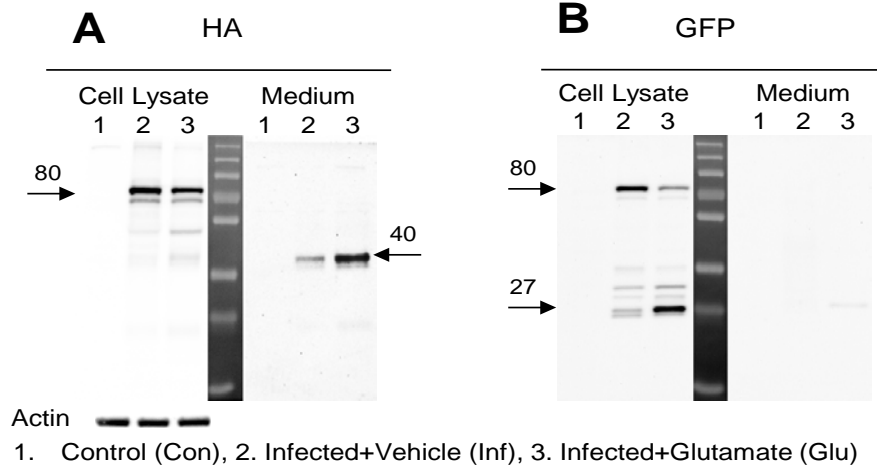
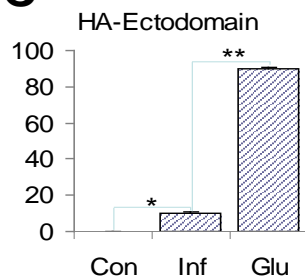
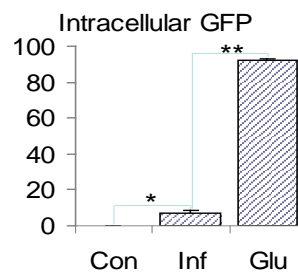
**C****D**

Fig. 2. The trauma-triggered vectors are activated in primary culture of cortical neurons. Lane 1 from the representative WB samples shows the cell lysate and media obtained from non-transfected neurons. Lane 2 shows the cell lysate and the media obtained from the AAV vector infected control neurons without treatment expressing only the full length construct. Lane 3 shows the lysate and the media obtained from the AAV vector infected neurons after 30 min glutamate (100 μ M) treatment. **(A)** The HA-tag labels the extracellular domain of the construct that is shed from the membrane and released into the medium, while **(B)** the GFP-tagged intracellular fragment is reduced in molecular weight to 27 kDa. **(C-D)** The panel shows the summary data from 3 repeats of the cell culture experiments of panel A and B respectively. The data confirm robust cleavage of the construct and release of HA-tagged ectodomain in the medium **(C)** and retention of GFP in the cytoplasm **(D)**.

PVR as an Experimental model of open globe ocular trauma

We have initiated *in vivo* studies to evaluate PVR as an experimental model of open globe ocular trauma (Agrawal et al., 2007) that will be used for testing and optimization of our gene therapy for ocular trauma *in vivo* (Fig. 3).

Accumulation of blood in the vitreous due to hemorrhage is the most important independent predictive factor for PVR following blunt and open globe ocular trauma. To simulate a trauma model of PVR, one of the eyes in rabbits was injured by a standard 8 mm circumferential scleral incision through the pars plana 2.5 mm from the limbus to the center of the vitreous cavity avoiding the lens and the peripheral retina. The prolapsed vitreous was abscised and the wound was closed with 8-0 silk sutures. At the end of the surgery, all eyes will receive 0.4ml of autologous blood, drawn immediately before the incision, injected through a 25-gauge needle inserted through the wound and into the mid vitreous under ophthalmoscopic control. These animals need to be followed for several months because the complete retinal detachment induced by PVR typically occurs after 6-8 weeks. The contralateral eye remained untouched to avoid any confounding effects, and also served as a contralateral control. The retinal status was evaluated with retinal imaging. Eyes were enucleated for histological and biochemistry analysis, IHC and WB to assess the extent retinal injury and its mechanisms including the expression and activation of metalloproteinases in the eye and the membrane shedding events after ocular trauma.

Rabbit PVR induction (with intraocular autologous blood injection)

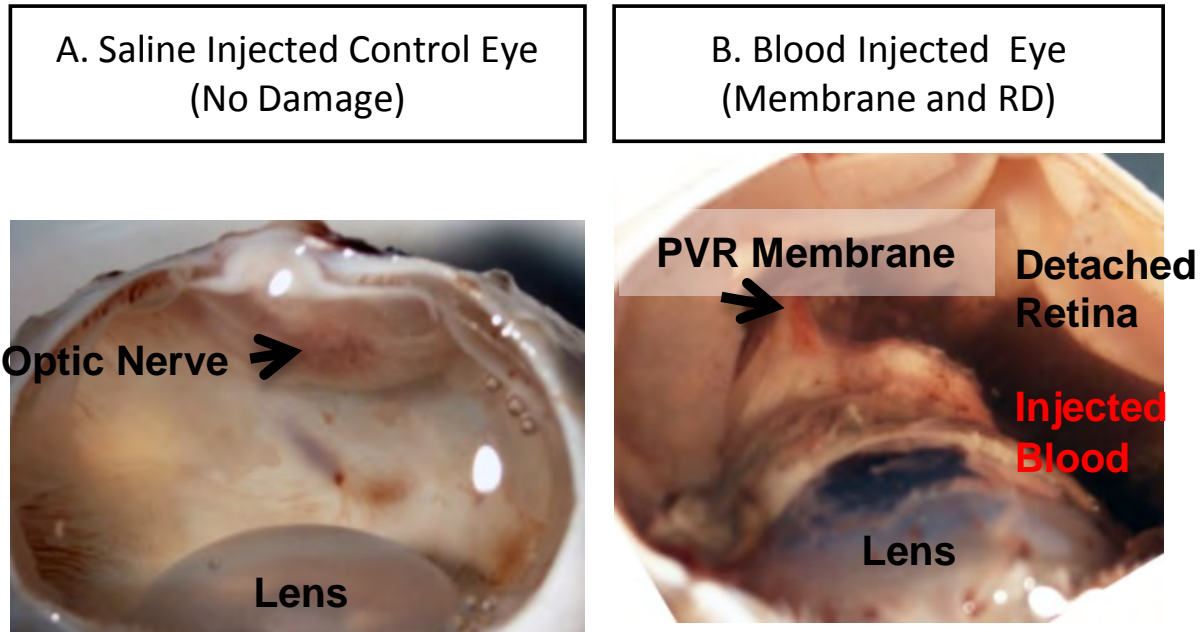


Fig. 3. Rabbit eye after PVR induction.

A. The saline injected eye (left) demonstrates a flat retinal surface, clear vitreous cavity, with lens and optic nerve visible.

B. The blood injected eye (right) demonstrates a detached bulging retinal surface (upper right), a significant membraneous growth attached to the retina (upper left, arrow). Remnants of autologous blood remain in vitreous cavity giving a darkened appearance to the vitreous chamber (right). RD, retinal detachment.

Since the effects PVR after penetrating ocular trauma will fully develop within 6-8 weeks, in addition to the time required for processing and analysis of isolated eye tissues, we anticipate to have some immunohistochemistry (IHC) data from our PVR model reported in the next reporting period.

What opportunities for training and professional development has the project provided?

Nothing to Report.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

To accomplish the goals and objectives from the approved SOW during the next reporting period we will continue to evaluate the expression and activation of MPs in the eye after trauma and assess their value as biomarkers of injury.

- We will continue the work on *in vivo* on the model of proliferative vitreoretinopathy (PVR) in rabbits. During the next reporting period of the project we will assess the metalloproteinase expression and activation after ocular trauma as potential biomarkers of injury.
- We will continue testing the reporter-tagged PAS constructs delivered by AAV *in vitro* using HEK293, neurons and eye specific cells.
- In the next reporting period we will develop PAS that will carry the therapeutic constructs as ectodomains. Expression of the tagged-PAS and the shedding of therapeutic ectodomains will be assessed in vitro using HEK293 cells.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report

6. PRODUCTS:

Publications, conference papers, and presentations

Nothing to Report

Website(s) or other Internet site(s)

Nothing to Report

Technologies or techniques

Nothing to Report

Inventions, patent applications, and/or licenses

Nothing to Report

Other Products

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

PDs/PIs on the project

Name:

Project Role:

Researcher Identifier (eRA ID):

Nearest person month worked:

Contribution to Project:

Dr. William Jia

PD/PI

WILLJIA

2

Dr. Jia was coordinating the preparation and submission of required regulatory documents, the development of PAS constructs and their evaluation *in vitro*.

Name:

Project Role:

Researcher Identifier (eRA ID):

Nearest person month worked:

Contribution to Project:

Dr. Max Cynader

Co-PD/PI

MAXCYNADER

1

Dr. Cynader was reviewing the required regulatory documents; he was overseeing the *in vitro* experiments used for testing the PAS constructs.

Name:
Project Role:
Researcher Identifier (eRA ID):
Nearest person month worked:
Contribution to Project:

Dr. Joanne Matsubara
Co-PD/PI
JOANNEMATSUBARA
1

Dr. Matsubara was directly involved in the preparation and review of the required regulatory documents and is coordinating the *in vivo* experiments modeling ocular trauma.

Name:
Project Role:
Researcher Identifier (eRA ID):
Nearest person month worked:
Contribution to Project:

Dr. Ljubomir Kojic
Co-Investigator
LJUBOMIRKOJIC
3

Dr. Kojic was directly involved in the designing of the PAS constructs; he was preparing the animal protocol and other regulatory documents, including the quarterly and yearly reports.

Name:
Project Role:
Researcher Identifier (eRA ID):
Nearest person month worked:
Contribution to Project:

Dr. Jing Cui
Co-Investigator
JINGCUI
3

Dr. Cui was directly overseeing and conducting the *in vivo* studies; she was involved in developing of the animal protocol and preparing of the quarterly and yearly reports.

Name:
Project Role:
Researcher Identifier (eRA ID):
Nearest person month worked:
Contribution to Project:

Luke Bu
Research Assistant
XUEXIANBU
2

Mr. Bu was involved in the development and testing of the AAV vectors and PAS constructs *in vitro*, the preparing of the animal protocol and the quarterly and yearly reports.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

No other partner organizations were involved.

8. SPECIAL REPORTING REQUIREMENTS

QUAD CHARTS:

We have updated and submitted the project Quad Chart as an attachment to the Quarterly Technical Report.

9. APPENDICES:

10. References:

- Agrawal RN, He S, Spee C, Cui JZ, Ryan SJ, Hinton DR (2007) In vivo models of proliferative vitreoretinopathy. *Nat Protoc* 2:67-77.
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Smart, Injury-triggered Therapy for Ocular Trauma

MR130584



PI: Dr. William Jia

Org: University of British Columbia

Award Amount: \$249,600

Study/Product Aim(s)

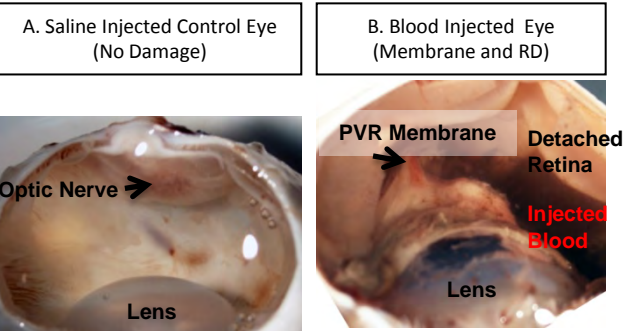
- To identify the metalloproteinases activated after ocular trauma.
- To develop and optimize genetically engineered trans-membrane proteins for instantaneous release of ectodomains in the vicinity of eye injury. To implement and optimize eye delivery methods using viral delivery strategies.
- To develop, optimize and validate “protease activity sensors” (PAS) able to deploy anti-inflammatory and neuroprotective “therapeutic ectodomains” (TE) in experimental models of PVR.

Approach

Our objective is to develop event-triggered gene therapy for PVR. We propose to engineer PAS able to locally release therapeutic ectodomains by metalloproteinases activated in PVR, to provide neuroprotection and prevent loss of vision. Our “smart therapeutics” will exploit the activation metalloproteinases to provide instantaneous and specific neuroprotection immediately after ocular traumatic injury, prevent PVR and avert loss of vision.

Rabbit PVR induction

(with intraocular autologous blood injection)



A. The saline injected eye (left) demonstrates a flat retinal surface, clear vitreous cavity, with lens and optic nerve visible. **B.** The blood injected eye (right) demonstrates a detached bulging retinal surface (upper right), a significant membranous growth attached to the retina (upper left, arrow). Remnants of autologous blood remain in vitreous cavity giving a darkened appearance to the vitreous chamber (right).

Timeline and Cost

Activities	CY	13	14	15	16
To obtain regulatory approval for animal model of ocular trauma and to identify metalloproteinases involved in eye injury					
To develop engineered transmembrane proteins that are shed and released in PVR and deliver them to the eye					
To evaluate and validate PAS for treatment of PVR after ocular trauma.					
Estimated Budget (\$K)				\$125	\$124.6

Goals/Milestones

CY14-15 Goals – To obtain local IACUC and ACURO approval for in vivo protocol that addresses animal model for ocular trauma

CY15-16 Goals – To identify the metalloproteinases activated after ocular trauma and to develop PAS and eye gene delivery vector

- ☐ Identify metalloproteinases activated after ocular injury using animal model of PVR
- ☐ Implement and optimize viral delivery system for the eye
- ☐ Develop PAS that are trauma-cleaved using *in vitro* and *in vivo* PVR models

CY16 Goals – To develop PAS carrying therapeutic domains (PAS-TE)

- ☐ Validate PAS and PAS-TE expression, and their *in vivo* therapeutic efficacy using experimental PVR model of ocular trauma.

Comments/Challenges/Issues/Concerns

- The timeline was adjusted to address the time for animal protocol approval by the local IACUC and DoD ACURO regulatory institutions.

Budget Expenditure to date

Projected Expenditure: \$249,600 Actual Expenditure: \$124,800

Updated: (Oct 14, 2015)